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1269069

THE UNITED STATES OF AMERICA

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APPLICATION NUMBER: *60/526,110*
FILING DATE: *December 01, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/40236*

UNITED STATES PATENT AND TRADEMARK OFFICE
DEPARTMENT OF COMMERCE

Certified By

Jon W Dudas

Under Secretary
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and Acting Director of the
Unites States Patent and Trademark Office

PROVISIONAL APPLICATION FOR PATENT COVER SHEET
Express Mail No. EL968313810US

	Docket Number	128034.100	Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Hanauske-Abel	H.	M.	Edgewater, NJ
Palumbo	P.		Westfield, NJ
Cracchiolo	B.	M.	Edgewater, NJ

TITLE OF THE INVENTION (280 characters max)

METHOD OF PREVENTING SURVIVAL OF RETROVIRALLY INFECTED CELLS AND OF INHIBITING FORMATION OF INFECTIOUS RETROVIRUSES

CORRESPONDENCE ADDRESS

Firm ID 21269

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification (Number of pages) [39] [ ] CDs (number) [ ]
[X] Drawings (Number of sheets) [1] [X] Other (specify): Postcard, Certificate of Mailing, Fee Transmittal and Check

METHOD OF PAYMENT (check one)

[X] Applicant(s) claim(s) small entity status. See 37 C.F.R. § 1.27. [X] A check or money order is enclosed to cover the Provisional filing fees [X] The Commissioner is hereby authorized to charge fees to Deposit Account Number: 50-0436	PROVISIONAL FILING FEE AMOUNT ($)	$80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[x] No [ ] Yes

[ ] Additional inventors are being named on separately numbered sheets attached hereto.

Respectfully submitted,

Raymond A. Miller

Raymond A. Miller
Registration No. 42,891

Date: December 1, 2003

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Compl te if Known	
Application Number	not yet assigned
Filing Date	December 1, 2003
First Named Inventor	Hanauske-Abel
Examiner Name	not yet assigned
Art Unit	not yet assigned
Attorney Docket No.	128034.100

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 50-0436

Deposit Account Name: Pepper Hamilton LLP

The Director is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					= 0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY

(Complete *(if applicable)*)

Name *(Print/Type)*	Raymond A. Miller	Registration No. *(Attorney/Agent)*	42,891	Telephone	412.454.5000
Signature	Raymond A. Miller			Date	December 1, 2003

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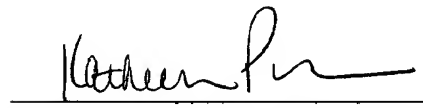
CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.10

APPLICANT:	H.M. Hanauske-Abel, et al.
TITLE:	METHOD OF PREVENTING SURVIVAL OF RETROVIRALLY INFECTED CELLS AND OF INHIBITING FORMATION OF INFECTIOUS RETROVIRUSES
SERIAL NO.:	NOT YET ASSIGNED
ATTORNEY REF:	128034.100
DATE OF DEPOSIT:	DECEMBER 1, 2003
EXPRESS MAIL NO.	EV968313810US

I HEREBY CERTIFY THAT THIS PROVISIONAL PATENT APPLICATION IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE VIA UNITED STATES POST OFFICE EXPRESS MAIL UNDER 37 C.F.R. § 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE MAIL STOP PROVISIONAL APPLICATION, COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450.

KATHLEEN PUJOL
(Typed or printed name of person mailing paper or fee)



(Signature of person mailing paper or fee)

DOCUMENTS ENCLOSED:

1. New Provisional Application Cover Sheet (1 sheet);
2. Provisional Application (39 pages);
3. Formal Figures (1 sheets)
3. Fee Transmittal and check in the amount of $80; and
4. Certificate of Mailing;
5. Postcard.

Inventors: H.M. Hanauske-Abel, et al.

METHOD OF PREVENTING SURVIVAL OF RETROVIRALLY INFECTED CELLS AND OF INHIBITING FORMATION OF INFECTIOUS RETROVIRUSES

BACKGROUND

[0001] Viruses require cells for their multiplication. In an infected cell, they achieve this goal by parsitizing and hijacking metabolic pathways, especially those involved in the synthesis of proteins and nucleic acids. Over evolutionary times, cells have developed mechanisms that detect the hijacking of these synthetic pathways by viral intruders. When triggered these mechanisms activate the invaded cell to initiate and complete self-destruction. The suicide of individual cells is intended to curb the spread of the viral intruder by destroying the production sites that release the invader in large numbers. All viruses therefore have developed molecules that inhibit the suicide of infected cells, in this way securing their continued enslavement as viral generators. Such antiapoptotic viral molecules are formed inside invaded cells as part of the viral takeover of the cellular synthetic machinery, which at the same time is also generating the infective viruses themselves.

[0002] Retroviruses use a particular set of viral proteins, of the Rev (SEQ ID NO:1)/Rex (SEQ ID NO:2) class, to transport certain viral mRNA from the nucleus of infected cells to the polysomes. These mRNAs display specific nucleotide motifs for binding to Rev (SEQ ID NO:1)/Rex (SEQ ID NO:2). Rev (SEQ ID NO:1)/Rex (SEQ ID NO:2)-dependent viral mRNAs are generally categorized as occurring late in the infections cycle, contain intronic sequences that otherwise would block their nuclear export, and encode major proteins essential for virion formation and suppression of apoptosis.

[0003] The cellular partner for the viral mediator proteins Rev (SEQ ID NO:1)or Rex (SEQ ID NO:2) is eukaryotic translation initiation factor 5A (eIF5A), of which two isoforms are known at present. Both contain the unique, genetically not encoded residue hypusine [N^{ε}-(4-amino-2(R)-hydroxybutyl)-L-lysine]. Hypusine is essential for the biological function of the eIF5A proteins, i.e. the nucleocytoplasmic transport of proliferation-related cellular mRNAs. Hypusine is formed through two consecutive posttranslational enzymatic modifications of a specific, genetically encoded lysine side chain of the eIF-5A precursor protein. In the first step, catalyzed by deoxyhypusine synthase (SEQ ID NO:6), the intermediate deoxyhypusine is formed by nicotinamide adenine dinucleotide (NAD)-dependent transfer of the 4 aminobutyl moiety of the polyamine spermidine to the ε-amino group of a specific lysine residue in the eIF-5A precursor protein. The last of these modifications is a hydroxylation, mediated by deoxyhypusine hydroxylase (DOHH (SEQ ID NO:5)), a 2-oxoacid utilizing dioxygenase like all other known protein hydroxylases. In HIV-infected cell lines, the 3-hydroxypyrid-4-one class of DOHH (SEQ ID NO:5) inhibitors deceases the formation of infective virions, disrupts the synthesis of the major capsid proteins, and induces apoptosis in the range of 100-200 μM.

SUMMARY

[0004] The present invention discloses compounds and pharmaceutical compositions which are highly effective at inhibiting the formation of the hypusine residue in cellular eIF-5A precursor proteins. The invention further relates to methods of using such compounds and pharmaceutical compositions therefrom for inhibiting or preventing the nucleocytoplasmic transport of viral polynucleotides from the nucleus of infected cells to cytoplasmic polysomes for

translation. Such inhibition appear to cause a dose-dependent initiation of apoptosis in the virally infected cells.

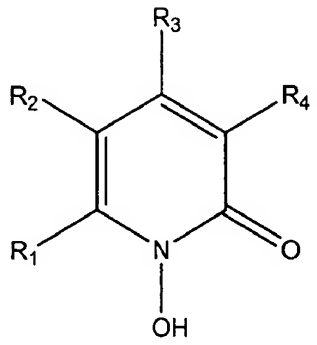
[0005] While not wishing to be bound by theory, it appears the structure and use of compounds of the present disclosed compounds prevent the viral take-over of the cellular synthetic machinery. The resulting disruption of viral protein formation causes loss of anti-apoptotic effect, resulting in reactivation of the self-destruction of cells. This compound and compositions also causes a general decrease in the viral proteins required for the assembly of infective viruses. The knowledge of the cellular molecules involved in the nucleocytoplasmic transport of proliferation-related cellular polynucleotides like viral mRNA, can be used for the rapid identification of compounds which may be used to reactivate the apoptotic clearance of virally infected cells. The administration of such compounds and composition can be employed clinically to break the cycle of viral infectivity.

[0006] The present invention is directed to therapeutic compositions and methods that employ compounds and composition that inhibit post-translational hypusine formation which thereby inhibits the formation of the intracelluar protein eIF-5A (SEQ ID NO:3). This inhibition may be used for suppressing infections by retroviruses that parasitize eIF-5A (SEQ ID NO:3) so as to promote their own replication. The methods of the present invention involves administering, to eukaryotic cells, tissues, or individuals, compound which blocks the post-translational intracellular formation of hypusine, in an amount effective to suppress biosynthesis of bioactive eIF-5A (SEQ ID NO:3) or its bioactive isoforms, suppress translational interaction of eIF-5A (SEQ ID NO:3) or its isoforms with retroviral elements of nucleic acid and/or protein structure, inhibit bonding or association of retroviral proteins of Rev (SEQ ID NO:1) and or Rex

(SEQ ID NO:2) viruses with eIF-5A (SEQ ID NO:3); inhibits formation of anti-apoptosis proteins from retroviral polynucleotides; and induce apoptosis of virally-infected cells.

[0007] One embodiment of the present invention involves inducing apoptosis in eukaryotic cells infected with Rev (SEQ ID NO:1) or Rex (SEQ ID NO:1) dependent retrovirus or viruses dependent on interaction of host cell eIF-5A (SEQ ID NO:3) with viral elements of nucleic acid and/or protein structure. This is achieved by administering an compound or composition to eukaryotic cells which blocks the post-translational intracellular formation of hypusine in an amount sufficient to induce apoptosis of virally-infected cells.

[0008] One embodiment of the present invention is a therapeutic composition comprised of a 1-hydroxy-2-pyridone compound of formula (1) and derivatives thereof (including salts, tautomeric forms, and solvates)or a pharmaceutical composition including the compound of formula (1),

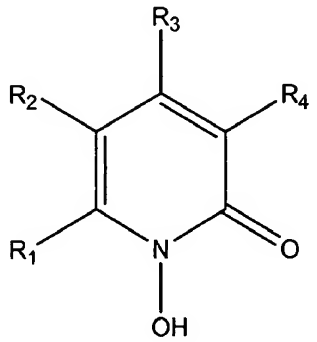


(I)

[0009] wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or

arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds. Examples of such compound and salts thereof of formula 1 useful in the practice of the present invention include but are not limited to rilopirox; ciclopirox, 6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone; its ethanolamine salt ciclopirox olamine; metipirox which is 1-hydroxy-4,6-dimethyl-2-(1H)-pyridone (CAS # 29342-02-7); piroctone (1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2-(1H)-pyridone [CAS # 506050-76-5]); and its (1:1) ethanol amine salt octopirox (CAS # 68890-6-4); 1-hydroxy-2-pyridones where R_3=Methyl, R_1, R_2, R_3= H; and 1-hydroxy-2-pyridones where R_1=Methyl, R_2, R_3, R_4= H.

[0010] Another embodiment of the present invention is a method of treating cells infected with a retrovirus that includes administering to eukaryotic cells a 1-hydroxy-2-pyridone compound of formula (1) and derivatives thereof (including salts, tautomeric forms, and solvates)or a pharmaceutical composition including the compound of formula (1),



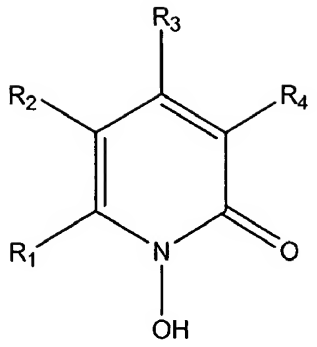
(I)

wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12

carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds. Examples of such compound and salts thereof of formula 1 useful in the practice of the present invention include but are not limited to rilopirox; ciclopirox, 6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone; its ethanolamine salt ciclopirox olamine; metipirox which is 1-hydroxy-4,6-dimethyl-2-(1H)-pyridone (CAS # 29342-02-7); piroctone (1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2-(1H)-pyridone [CAS # 506050-76-5]); and its (1:1) ethanol amine salt octopirox (CAS # 68890-6-4); 1-hydroxy-2-pyridones where R_3=Methyl, R_1, R_2, R_3= H; and 1-hydroxy-2-pyridones where R_1=Methyl, R_2, R_3, R_4= H.

[0011] The one or more cells to which the compound or compositions are administered may be present *in vivo* or *in vitro* in a fluid, a tissue, an organ, or in an individual. The administration may include cycled (high) dose (pulse) administrations of the compound. The viral mRNA present in the cell may be transported to polysomal ribosomes of said cell by the cellular transport protein eIF-5A. The virus may be a lentivirus.

[0012] One embodiment of the present invention is a method of inhibiting viral replication in cells which may include administering to eukaryotic cells or tissues including such cells, a composition including a compound described herein and preferably a compound of formula (I) and derivatives thereof,



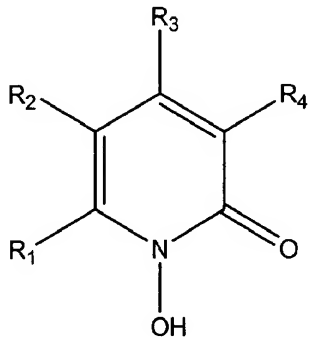
(I)

wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds. The compound of formula (1) or composition inhibits and interferes with the formation of the transport eIF-5A (SEQ ID NO:3) protein capable of bonding with a viral mediator Rev (SEQ ID NO:1) and or Rex (SEQ ID NO:2) proteins. These proteins, which are encoded by lentiviruses and other complex retrovisus, mediate the nucleocytoplasmic transport of intron-containing, viral pre-mRNAs with the transport protein eIF-5A (SEQ ID NO:3)) within the cells.

[0013] The Rev (SEQ ID NO:1) viral mediator protein preferably interacts with the RRE nucleotide sequence of the viral mRNA; the Rex (SEQ ID NO:2) viral mediator protein preferably interacts with the RxRE nucleotide sequence of the viral mRNA. The compound or composition administered to the one or more cells interferes with the deoxyhypusine hydroxylase mediated formation of a hydropusine residue on an eIF-5A precursor protein. The

administration may include cycled doses or pulse administrations of the composition or compound of Formula (1).

[0014] One embodiment of the present invention is a method of modifying apoptosis in cells comprising: administering to eukaryotic cells, tissues, or individuals a composition including a compound of formula (1),

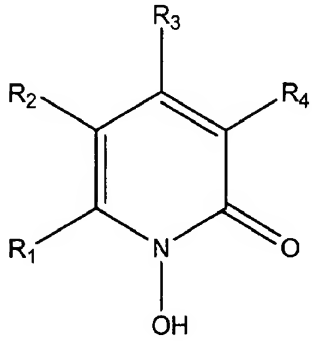


(I)

wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds. The cells include transcribed viral mRNA that encode proteins to suppress the apoptosis of the cells. The compound or composition of formula (1) inhibits the formation of a complex between the complex of the viral mRNA and a mediator protein such as Rev (SEQ ID NO:1) or Rex (SEQ ID NO:2) and the transport protein eIF-5A (SEQ ID NO:3)) and any of its isoforms.

[0015] Preferably the compound or compositions being utilized in the present invention inhibits the formation of a hypusine residue on a transport protein precursor eIF-5A of the cell, and prevents the biologically active transport protein eIF-5A from interacting with viral mRNA in the nucleus and exporting it to the the cytoplasmic polysomes for translation. The compound or compositions may be administrated and include cycled doses.

[0016] One embodiment of the present invention is a method of treating cells infected with a retrovirus that includes the acts of identifying eukaryotic cells having a retrovirus that uses the eIF-5A (SEQ ID NO:3) cellular protein to transport retroviral m RNA from the nucleus of the cells to the cytoplasmic polysomes (includes ribosomes) of the cells and administering to the infected cells a compound or composition that includes the compound of formula (I) and derivatives thereof,



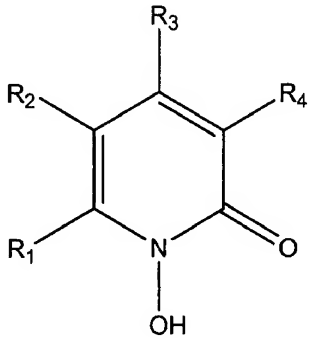
(I)

wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or

peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds.

[0017] The cells may be *in vitro* or *in vivo* and present in a fluid, a tissue, or in an individual. The retroviral mRNA may be transported to polysomal ribosomes of the cell through the translation initiation factor eIF-5A. The administration may include cycled doses, including high doses of the compound or compositions including the compound of formula (1).

[0018] One embodiment of the present invention is a method of treating cells infected with a retrovirus that includes identifying eukaryotic cells at risk of being infected with a virus that uses the eIF-5A (SEQ ID NO:3) cellular protein to transport viral m RNA from the nucleus of the cells to the cytoplasmic polysomes of the cells and administering to the cells a composition including a compound of formula (I) and derivatives thereof,

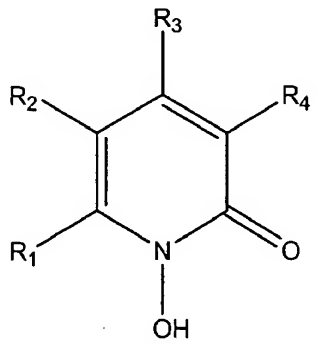


(I)

wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or

amide of any of the compounds. One or more of the cells may be present in a tissue or in an individual. The viral mRNA in the nucleus of the cell is preferably transported to polysomal ribosomes in the cytoplasam of the cell. The administration may include cycled (high) dose (pulse) administrations of the composition.

[0019] A method of treating cells infected with a retrovirus that include administering to eukaryotic cells having a viral mRNA in the nucleus of the cells a composition including the compound of formula (I) and derivatives thereof,



(I)

[0020] wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds and inhibiting at least a portion of deoxyhypusine hydroxylase present in the cells.

DESCRIPTION OF THE DRAWINGS

[0021] In part, other aspects, features, benefits and advantages of the embodiments of the present invention will be apparent with regard to the following description, appended claims and accompanying drawings where

[0022] FIG. 1 includes non-limiting illustrations of compounds useful in the practice of the present invention;

DETAILED DESCRIPTION

[0023] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0024] It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned

herein are incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0025] The present invention is directed to methods of inhibiting the post-translational formation of the genetically non-coded residue hypusine [N^{ε}-(4-amino-2(R)-hydroxybutyl)-L-lysine] to form the cellular protein eukaryotic initiation factor-5A (eIF-5A (SEQ ID NO:3). More particularly, the present invention involves inhibiting intracellular synthesis of functional bioactive eIF-5A (SEQ ID NO:3), inhibiting the translationally productive interaction of eIF-5A (SEQ ID NO:3) with complexes of nucleic acids and/or viral mediator proteins structure and inhibiting transport of viral polynucleotides to ribosomes in the cytoplasm. These methods include administering, to eukaryotic cells, tissues, or individuals, an composition which inhibits in a sufficient amount the post-translational intracellular formation of hypusine from an eIF-5A protein precursor. Preferably the composition is a deoxyhypusyl hydroxylase synthase (SEQ ID NO: 6) inhibitor in an amount sufficient to suppress the translationally productive interaction of eIF-5A (SEQ ID NO:3) with viral elements of nucleic acid and/or protein structure in the nucleus of the one or more cells.

[0026] Another aspect of the present invention involves inducing apoptosis in eukaryotic cells infected with Rev (SEQ ID NO:1) or Rex (SEQ ID NO:2) dependent retroviruses or lentiviruses dependent on interaction of host cell eIF-5A (SEQ ID NO:3) with viral elements of nucleic acid and/or protein structure which encode proteins to inhibit apoptosis. Inducing apoptosis in these infected cells is achieved by administering a compound of formula (1) to eukaryotic cells infected with Rev (SEQ ID NO:1) or Rex (SEQ ID NO:2) dependent retroviruses or lentiviruses dependent on interaction of eIF-5A (SEQ ID NO:3) with viral elements of nucleic acid and/or protein structure. The composition of formula (1) inhibits or

preferably blocks the post-translational intracellular formation of hypusine in an amount sufficient to prevent transport of viral mRNA encoding apoptosis inhibiting proteins from translation thereby inducing apoptosis of virally-infected cells.

[0027] Retroviruses, of which lentiviruses are a genus, are typified by the human immunodeficiency virus type 1 (HIV-1), share the strict requirement for a specific regulator (i.e. protein and/or nucleic acid) in order to express viral structural genes and, hence, to propagate efficiently and produce infectious progeny. In addition to the human immunodeficiency viruses, this group consists of, but is not not limited to, human T-cell leukemia viruses, hepatitis B virus, visna virus, simian immunodeficiency viruses, bovine immunodeficiency virus, equine infectious anemia virus, feline immunodeficiency viruses, caprine arthritis-encephalitis virus, and Mason-Pfizer virus. Reference to HIV-1 is used here as a non-limiting example to to exemplify the function of this specific regulator, to delineate its interaction with host cell eIF-5A (SEQ ID NO:3), and to demonstrate the methods of this invention as they are applied to interfere with this specific regulator and render it nonfunctional.

[0028] Retroviruses use a particular set of viral proteins, of the Rev (SEQ ID NO:1) and or Rex (SEQ ID NO:2) class, to transport certain viral mRNA from the nucleus of infected cells to the polysomes in the cytoplasm of the cell. These mRNAs display specific nucleotide motifs for binding to the viral mediator proteins Rev (SEQ ID NO:1) and or Rex (SEQ ID NO:2). Rev (SEQ ID NO:1) and orRex (SEQ ID NO:2)-dependent viral mRNAs are generally categorized as occurring late in the infections cycle, contain intronic sequences that otherwise would block their nuclear export, and encode major proteins essential for virion formation and suppression of apoptosis.

[0029] Retrovirus any of a family (Retroviridae) of single-stranded RNA viruses (as HIV and the Rous sarcoma virus) that produce reverse transcriptase by means of which DNA is synthesized using their RNA as a template and incorporated into the genome of infected cells and that include numerous tumorigenic viruses -- called also RNA tumor virus

[0030] With respect to the present inventive methods, the tissue can be a tissue of any living mamal and may be *in vivo* or *in vitro*. Mammals may include, but are not limited to, the order Rodentia, such as mice, and the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0031] Furthermore, the tissue can be a tissue of any type, such that the tissue comprises cells of any type. However, it is preferred that the tissue is a tissue of an epithelial origin.

[0032] Particularly, it is preferred that the tissue is a surface epithelium. Moreover, the tissue of the present inventive methods can be in vivo or ex vivo. The term"in vivo"as used herein means that the tissue is found within a living system. The term"ex vivo"as used herein means that the tissue is derived from a living system but is taken out of the living system.

[0033] After viral infection of human cells by a retrovirus such as but not limited to HIV-1, the viral genomic RNA is transcribed into DNA and subsequently incorporated into the human genome. Upon transcription, only the completely spliced about 2-kb transcripts encoding the HIV-1 proteins, Tat, Rev (SEQ ID NO:1), and Nef, or Tat-Rev (SEQ ID NO:1) fusion proteins, are exported to the cytoplasm for efficient translation by the protein producing machinery of the

host cell. The incompletely spliced about 4-kb and the unspliced about 9-kb viral transcipts are not themselves exported and, thereby, fail to gain productive access to this machinery, apparently due to control mechanisms that in eukaryotes generally deny translation of incompletely spliced and unspliced RNA. This failure to be exported, apparently due to lack of nucleocytoplasmic transport and/or polysomal translation, is of grave consequence to the replicative ability of retrovirusis like HIV-1 and severely limits production of new virions. Not only are all the structural proteins of the HIV-1 particle encoded by these incompletely/unspliced transcripts, but the about 9-kb species also constitutes the infectious viral genome to be packaged into these particles. It is the function of the Rev (SEQ ID NO:1) protein, the specific regulator of HIV-1, to enter into the nucleus after being synthesized on cytoplasmic host cell polysomes, to bind to the Rev (SEQ ID NO:1)-response element ("RRE") of the about 4-kb and about 9-kb transcripts, and, thereby, to convey them to the protein producing machinery of the host cell for effective biosynthesis of the viral proteins, Gag, Pol, Vif, Vpr, Vpu, and Env.

[0034] The Rex (SEQ ID NO:2) mediator protein encoded by the human T-cell leukemia virus group of retroviruses binds to the nucleotide element RxRE of HTLV-1.

[0035] Rev (SEQ ID NO:1) is known to bind to the eIF-5A (SEQ ID NO:3) of infected host cells ›Ruhl et al., J. Cell. Bio. 123, 1309-1320 (1993), which is hereby incorporated by reference. The eIF-5A (SEQ ID NO:3) protein is the critical element in a proposed pathway to provide preferential polysomal access to a subclass of specific cellular mRNAs which encode proteins that enable and coordinate DNA replication, i.e., initate cellular proliferation. eIF-5A (SEQ ID NO:3) is unique in that it is the only protein known to containing a lysine-derived hypusine residue which is formed post-translationally by the enzymes deoxyhypusyl synthase DOHS (SEQ ID NO:6) and deoxyhypusyl hydroxylase DOHH (SEQ ID NO:5). Once hypusine

is formed, eIF-5A (SEQ ID NO:3) physically interacts with a small subclass of the total cellular mRNAs; this subset has been termed hypusine-dependent messenger nucleic acids, or hymns (Hanauske-Abel et al., FEBS Lett. 366, 92-98 (1995)), which is hereby incorporated by reference. In this way, eIF-5A (SEQ ID NO:3) enables preferential polysomal loading of the estimated only about 120 different mRNAs of the hymn type and directly entitles them to translation, bypassing the need to "wait in line" until ribosomes become available. The hymn-encoded proteins, in turn, are essential for irreversibly engaging the multi-component machinery that initiates replication of eukaryotic cells (Hanauske-Abel et al., FEBS Lett. 366, 92-98 (1995)), which is hereby incorporated by reference. Translation of the vast majority of cellular mRNAs, estimated to reach over 20,000 distinct species per cells is bypassed. The routine translation of all these mRNAs constitutes the usual mechanism for "household" protein biosynthesis and proceeds independent of hypusine formation and the eIF-5A (SEQ ID NO:3) pathway.

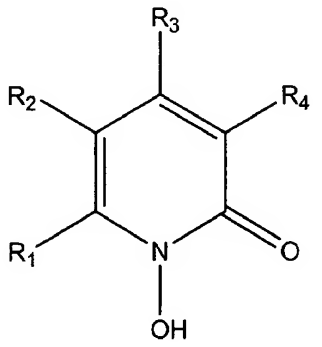
[0036] Tthe eIF-5A (SEQ ID NO:3) pathway can be parasitized by the human immunodeficiency virus type 1 ("HIV-1") ›Ruhl et al., J. Cell. Biol. 123, 1309-1320 (1993), which is hereby incorporated by reference. HIV-1 now is being recognized as a non-limiting representative example of the class of viruses that, after penetration into eukaryotic cells, feed on the eIF-5A (SEQ ID NO:3) pathway to achieve preferential translation of viral structural proteins and thus, gain a generative advantage. This compatible with the finding that HIV-1 multiplication occurs preferentially in proliferating cells, particularly of the T-cell lineage ›see, for instance, Gowda et al., J. Immunol. 142, 773-780 (1989) or Klatzmann et al., Immunol. Today 7, 291-296 (1986), and references therein, all of which are hereby incorporated by reference, and is compatible with the finding that efficient HIV replication in human peripheral

blood mononucleolar cells and in human T-cell lines correlates with eIF-5A (SEQ ID NO:3) expression (Bevec et al., Proc. Natl. Acad. Sci. USA 91, 10829-10833 (1994), which is hereby incorporated by reference. A subclass of viral mRNAs encoding in particular the structural proteins that form the virion core and capsid, interacts with the viral protein Rev (SEQ ID NO:1) through a specific nucleotide sequence, the Rev (SEQ ID NO:1) response element ("RRE"). The Rev (SEQ ID NO:1)/RRE unit constitutes the specific regulator for biosynthesis of HIV-1 proteins (see, for instance, Gallo et al., The Human Retroviruses, 69-106, Academic Press (1991), which is hereby incorporated by reference. It is the Rev (SEQ ID NO:1) component of this complex which specifically interacts with host cell eIF-5A (SEQ ID NO:3) (Ruhl et al., J. Cell. Biol. 123, 1309-1320 (1993), which is hereby incorporated by reference. As a result, these RRE-containing viral mRNA species, which otherwise would show very limited or no translational efficiency, become eligible for preferential polysomal loading and translation, resulting in active production of infective HIV-1 virions. In this manner, the production of key proteins for virion formation and packaging is assured and viral replication guaranteed. Production of Rev (SEQ ID NO:1) at the host cell polysomes is known to occur independent of RRE, Rev (SEQ ID NO:1), and eIF-5A (SEQ ID NO:3).

[0037] The cellular partner for Rev (SEQ ID NO:1)/Rex (SEQ ID NO:2) is eukaryotic translation initiation factor 5A (eIF5A), of which two isoforms are known at present. Both contain the unique, genetically not encoded residue hypusine. Hypusine is essential for the biological function of the eIF5A proteins, i.e. the nucleocytoplasmic transport of proliferation-related cellular mRNAs. Hypusine is formed through two consecutive posttranslational modifications of a specific, genetically encoded lysine side chain. The last of these modifications is a hydroxylation, mediated by deoxyhypusine hydroxylase (DOHH (SEQ ID

NO:5)), a 2-oxoacid utilizing dioxygenase like all other known protein hydroxylases. In HIV-infected cell lines, the 3-hydroxypyrid-4-one class of DOHH (SEQ ID NO:5) inhibitors deceases the formation of infective virions, disrupts the synthesis of the major capsid proteins, and induces apoptosis in the range of 100-200 μM.

[0038] Compositions of the present invention include a compound of formula (1) and derivatives thereof (salts, solvates, tautomeric forms),



(I)

wherein R_1, R_2, R_3, and R_4 can be the same or different and each is independently a hydrogen; an alkyl, alkenyl, or alkoxy group, any of which contains 1 to about 8 carbon atoms; an aryl, carbamyl, aminocarbonyl, aralkyl, or cycloalkyl group, any of which contains 5 to about 12 carbon atoms; an alkoxycarbonyl, carbamyl, aminocarbonyl, alkylaminocarbonyl, or arylaminocarbonyl group, any of which contains up to about 15 carbon atoms; a peptide or peptidomimetic moiety, either of which contains 10 to about 100 carbon atoms; a salt, ester, or amide of any of the compounds as disclosed in WO 03/018014 the contents of which are incorporated herein by reference in their entirety.. Examples of such compound and salts thereof

of formula 1 useful in the practice of the present invention include but are not limited to rilopirox; ciclopirox, 6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone; its ethanolamine salt ciclopirox olamine; metipirox which is 1-hydroxy-4,6-dimethyl-2-(1H)-pyridone (CAS # 29342-02-7); piroctone (1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2-(1H)-pyridone [CAS # 506050-76-5]); and its (1:1) ethanol amine salt octopirox (CAS # 68890-6-4); 1-hydroxy-2-pyridones where R_3=Methyl, R_1, R_2, R_3= H; and 1-hydroxy-2-pyridones where R_1=Methyl, R_2, R_3, R_4= H .

[0039] These compounds may be made by the methods disclosed in U.S. Pat. No. 2,540,218 and U.S. Pat. No. 4,797,409 the contents of which are incorporated herein by reference in their entirety.

[0040] The hydroxypyridones of the present invention may be used in the free form or as their physiologically tolerated salts with inorganic or organic bases such as but not limited to NaOH, KOH, $Ca(OH)_2$, NH_3, and $H_2NCH_2CH_2OH$.

[0041] For purposes of all of the present inventive methods, the amount or dose of the compound administered should be sufficient to effect a therapeutic response in the animal over a reasonable time frame. Particularly, the dose of any composition including one or more of the compounds of any of Formula [1-4] should be sufficient to inhibit formation hypusine in precursor protein of eIF-5A within about 24 hours. The dose will be determined by the efficacy of the particular compound and the condition of the patient, tissue, or cell sample as well as the mass of the sample or patient to be treated. Many assays for determining an administered dose are known in the art. For purposes of the present invention, an assay, which comprises comparing the extent to which viral proliferation is inhibited in a tissue or sample of cells upon administration of a given dose of a compound to set of samples that are each given a different

dose of the compound, could be used to determine a starting dose to be administered. The extent to which cell apoptosis is restored upon administration of a certain dose can be assayed as known to those skilled in the art and described herein.

[0042] The size of the dose also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular compound. Ultimately, the attending physician will decide the dosage of the compound of the present invention with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, inhibitor to be administered, route of administration, and the severity of the condition being treated.

[0043] One skilled in the art will appreciate that suitable methods of administering a compound of the present invention are known, and, although more than one route can be used to administer a particular composition, a particular route can provide a more immediate and more effective response than another route.

[0044] Formulations suitable for oral administration of compositions which include compound of the present invention can consist of (a) liquid solutions, such as an effective amount of the compounds dissolved in diluents, such as water or saline, (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solids or granules, (c) suspensions in an appropriate liquid, and (d) suitable emulsions.

[0045] Tablet forms can include one or more of lactose, mannitol, cornstarch potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose, sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and

acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

[0046] Formulations of the compounds of formula (1-4) in compositions suitable for parenteral administration include aqueous and non- aqueous solutions, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0047] With respect to all of the present inventive methods, the 1-hydroxy-2-pyridones such as ciclopirox can be administered topically, systemically (intraveneously or subcutaneously).

[0048] Furthermore, all of the present inventive methods can comprise the administration of the compound, in the presence or absence of an agent that enhances its efficacy, or the methods can further comprise the administration of other suitable components, such as radiation therapy or chemotherapy with another active agent. The term "radiation therapy"as used herein refers to the treatment of disease (especially cancer) by exposure to radiation. The term"chemotherapy"as used herein refers to the treatment of cancer using specific chemical agents or drugs that are destructive of malignant cells and tissues. Chemotherapy refers to the

treatment of disease using chemical agents or drugs that are toxic to the causative agent of the disease, such as a virus, bacterium, or other microorganism.

[0049] Compounds of the present invention may be linked to radiological moieties such as ^{125}I for treatment of specific cancers.

[0050] If combined with radiation therapy or chemotherapy, the compounds of the present invention can be administered simultaneously or sequentially. The term "sequentially" as used herein refers to the compound being administered either before or after the radiation therapy or chemotherapy. Preferably, the compound is administered first, particularly if combined with radiation therapy.

[0051] One of ordinary skill in the art will readily appreciate that each compound of the present inventive methods can be modified in any number of ways, such that the therapeutic efficacy of the compound is increased through the modification. For instance, the compound could be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds to targeting moieties is known in the art. See, for instance, Wadwa et al., J. Drug Targeting 3: 111 (1995), U. S. Patent No. 5,087, 616, and U.S. Pat. No. 5,849,587 the contents of which are included herein by reference in their entirety. The term"targeting moiety"as used herein, refers to any molecule or agent that specifically recognizes and binds to a cell-surface receptor, such that the targeting moiety directs the delivery of the compound to a population of cells on which surface the receptor is expressed. Targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, hormones, growth factors, cytokines, and any other naturally-or non- naturally-existing ligands, which bind to cell surface receptors. The term"linker"as used herein, refers to any agent or molecule that bridges the compound to the targeting moiety.

[0052] One of ordinary skill in the art recognizes that sites on the compounds, which are not necessary for the function of the compound, are ideal sites for attaching a linker and/or a targeting moiety, provided that the linker and or targeting moiety, once attached to the compound does not interfere with the function of the compound, i.e. the ability to inhibit formation of the hypusine residue and form eIF-5A.

[0053] Alternatively, the compounds of the present invention can be modified into a depot form, such that the manner in which the compound is released into the body to which it is administered is controlled with respect to time and location within the body (see, for example, U.S. Patent No. 4,450, 150). Depot forms of compounds can be, for example, an implantable composition comprising the compound and a porous material, such as a polymer, wherein the compound is encapsulated by or diffused throughout the porous material. The depot is then implanted into the desired location within the body and the compound is released from the implant at a predetermined rate by diffusing through the porous material.

[0054] The compounds of the present invention can be used to treat a number of viral diseases caused by viruses that require a specific mediator protein (i.e. Rev (SEQ ID NO:1) or a functional equivalent) to express viral structural genes and to propagate efficiently. Such viruses include, but are not limited to, the lentiviruses pathogenic for humans and animals, in particular the human, bovine, feline, and simian immunodeficiency viruses, the equine infectious anemia virus, the caprine arthritis-encephalitis virus, and the visna virus.

[0055] In the practice of the present invention, compositions that include compounds o of forumula (1) can be administered topically or systemically. More particularly, such administration can be orally; parenterally, i.e. by subcutaneous, intravascular, or intramuscular injection; intraperitoneally; intrathecally; or by topical application, e.g. to skin or eyes, or by

application to the mucous membranes of the nose, throat, bronchial tree, or rectum, etc. They may be administered alone or with suitable pharmaceutical carriers, and can be in solid or liquid form such as tablets, capsules, powders, solutions, suspensions, or emulsions. The dosage of the active compound depends on the species of warm-blooded animal, the body weight, age, and mode of administration.

[0056] The pharmaceutical products of the present invention are prepared by dissolving, mixing, granulating, or tablet-coating processes known to those skilled in the art. For oral administration, the active compounds or their physiologically tolerated derivatives such as salts, esters, or amides, are mixed with the additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and are converted by customary methods into a suitable form for administration, such as tablets, coated tablets, hard or soft gelatin capsules, aqueous, alcoholic, or oily suspensions, or aqueous, alcoholic or oily solutions. Examples of suitable inert vehicles are conventional tablet bases such as lactose, sucrose, or cornstarch in combination with binders like acacia, cornstarch, gelatin, or with disintegrating agents such as cornstarch, potato starch, alginic acid, or with a lubricant like stearic acid or magnesium stearate. Examples of suitable oily vehicles or solvents are vegetable or animal oils, such as sunflower oil or fish-liver oil. Preparations can be effected both as dry and as wet granules.

[0057] For parenteral administration (subcutaneous, intravascular, or intramuscular injection), the active compounds or their physiologically tolerated derivatives such as salts, esters, or amides, are converted into a solution, suspension, or emulsion, if desired, with the substances customary and suitable for this purpose, such as solubilizers or other auxiliaries. Examples are: sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. Illustrative oils are those of petroleum, animal,

vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

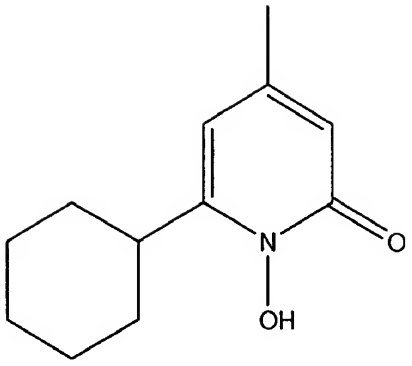
[0058] For use as aerosols, the active compounds or their physiologically tolerated derivatives such as salts, esters, or amides, may be dissolved or suspended in a physiologically acceptable liquid and packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The agents which block intracellular hypusine formation, in accordance with the present invention, may also be administered from a non-pressurized container such as a nebulizer or atomizer.

[0059] For topical administration to external or internal body surfaces, e.g., in the form of creams, gels, or drops, etc., the active compounds or their physiologically tolerated derivatives such as, salts, esters, or amides, are prepared and applied as solutions, suspensions, or emulsions in a physiologically acceptable diluent with or without a pharmaceutical carrier.

[0060] Various aspects of the present invention will be illustrated with reference to the following non-limiting examples.

EXAMPLE 1

[0061] This example demonstrates that the 1-hydroxy-2-pyridone ciclopirox, formula (2)



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is about ten times more potent in causing a very similar spectrum of effects. Ciclopirox, is representative for the entire class of 1-hydroxy-2-pyridones, for instance with regard to their ability to form high-spin 1:3 complexes with d^2sp^3-hybridized metals. Test results repeatedly demonstrate that ciclopirox, causes a dose-dependent initiation of apoptosis in the HIV-1 infected lymphocyte cell line H9.

[0062] Exposure of these HIV-1 infected lymphocyte cell line H9 cells for a mere 18 hours (overnight) at 40 μM of (2) causes apoptosis in more tha 30% of all cells. This effect is accompanied by a dose-dependent inhibition of cellular DOHH (SEQ ID NO:5). By contrast, the agent does not trigger apoptosis in HPV infected human keratinocytes or in native human lymphocytes not infected by HIV-1. In these latter two cases, the virally infected and the normal cells are not kept alive by anti-apoptotic proteins whose nucelocytoplasmic transport and polysomal translation depend on a Rev (SEQ ID NO:1)- and therefore eIF-5A (SEQ ID NO:3)/DOHH (SEQ ID NO:5)-dependent mechanism.

[0063] The results show that selective ablation of HIV-1 infected cells may be achieved by disrupting the translation of retroviral anti-apoptotic proteins that are encoded by Rev (SEQ

ID NO:1)-dependent mRNAs shuttled to those cell polysomes via the eIF-5A (SEQ ID NO:3) pathway of nuclear export.

[0064] Systemic application of the 1-hydroxy-2-pyridones may be used to treat cells, tissues, or patients having tissues with such viruses. The compositions may include the compound of formula (1) and their derivatives. The application may include cycled high dose pulse therapy administration of the composition to the cells, tissue, or patient to achieve ablation of lentiviruses, retroviruses, and preferably HIV infected cells throughout the body. Repeated treatment cycles with 1-hydroxy-2-pyridonense and their derivatives is possible, as are combinations with currently available chelators like desferal, to maximize the amount of a 1-hydroxy-2-pyridone like ciclopirox reaching DOHH (SEQ ID NO:5), and to minimize the amount lost due to non-specific binding to metal ions, and or the currently available antiretrovirals, since none of the latter acts as an inhibitor of DOHH (SEQ ID NO:5)/ the eIF-5A (SEQ ID NO: 3) pathway.

EXAMPLE 2

[0065] This prophetic example illustrates use of the compound of the present invention in compositions.

[0066] The compounds of the present invention may be included in compositions for topical applications. The 1-hydroxy-2-pyridones may be admixed into spermicidal creames, or coated into the lubricant of condoms, or used in preparations intended for pre/post-coital application. In such compositions the 1-hydroxy-2-pyridones may limit the production of infectious virons by lymphocytes in the ejaculate. Such lymphocytes appear to play a decisive role in genital transmission fo HIV-1 which is premised on the fact that lymphocyte removal from the sperm of HIV-1 positive males during processing for *in vitro* fertilization results in non-

infectious semen, as evidenced clinically by absent infection if inseminated females as well as by the generation of entirely healthy babies.

EXAMPLE 3

[0067] This example illustrates the selective ablation of HIV-infected lymphocytes by inhibitors of hypusine formation.

[0068] Mature eIF5A, involved in nucleocytoplasmic transport of certain mRNAs, contains the functionally essential residue hypusine. The latter is formed by deoxyhypusine hydroxylase (DOHH (SEQ ID NO:5)), a 2-oxoacid utilizing non-heme iron dioxygenase whose catalysis follows the HAG mechanism (Hanauske-Abel et al., Curr Med Chem 10: 1037-1050 (2003)). Mature eIF5A is a cellular cofactor of the viral Rev (SEQ ID NO:1)/Rex (SEQ ID NO:2) proteins (Hauber, Curr Top Microbiol Immunol 259:55-76 (2001)), required for retroviral multiplication and suppression of host cell apoptosis. It may be that HAG mechanism-based agents that target the ferrous ion of DOHH (SEQ ID NO:5), but not metal chelators in general, deny mature eIF5A to HIV-1, causing a lack of retroviral anti-apoptotic proteins and thus releasing the self-destruction of HIV-infected lymphocytes. DOHH (SEQ ID NO:5) inhibition in human papillomavirus (HPV)-infected or normal cells should not trigger apoptosis.

[0069] Methods: The drug deferiprone (DEF) and ciclopirox (CPX), known as DOHH (SEQ ID NO:5) inhibitors, were compared to the chelators 2-imidazoyl-4-methylphenol (IMP) and desferal (DES). Apoptosis of HIV-1 infected H9 cells, HPV-16 infected SiHa cells, and uninfected lymphocytes was analyzed by TUNEL flow cytometry. DOHH (SEQ ID NO:5) activity was measured by metabolic labeling with 3H-spermidine.

[0070] Results: In a dose-dependent manner, DEF and CPX inhibited DOHH (SEQ ID NO:5) in HIV-positive H9 cells and in HPV-positive SiHa cells. The chelator IMP was

uniformly ineffective even at 400 μM. In the H9 cells, only the DOHH (SEQ ID NO:5) inhibitors DEF and CPX triggered dose-dependent apoptosis. The chelators IMP and DES failed to elicit apoptosis even at maximal concentrations (400 μM and 20 μM respectively). With complete suppression of DOHH (SEQ ID NO:5) activity, i.e. at 200 μM DEF or 40 μM CPX, at least 30% of these H9 cells became apoptotic within 20 hours. By contrast, 40 μM CPX did not initiate apoptosis in the SiHa cells even after 120 hours, although CPX totally suppressed their DOHH (SEQ ID NO:5) activity. Likewise, lymphocytes harvested from health volunteers failed to respond with apoptosis when exposed for 20 hours to DEF, CPX, or DES.

[0071] The results demonstrate the ability to chelate iron in solution is not sufficient for DOHH (SEQ ID NO:5) inhibition. The latter triggers apoptosis in HIV-infection, but not in HPV-infected or normal cells. Deferiprone is a clinical trail candidate for a novel treatment strategy: Cycled, high-dose pulse therapty to achieve selective ablation of infected cells, bypassing all currently pursued and mutation-proneviral targets.

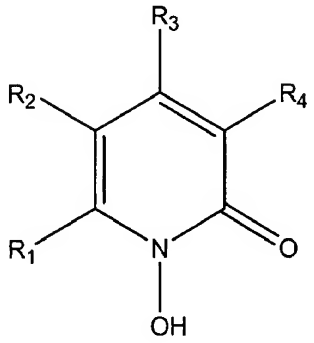
[0072] Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. For example While not wishing to be bound by theory, since the compounds of the general formula (1), according to this invention, inhibit the enzymatically catalyzed hydroxylations of proteins, they are apt to prevent the maturation of such proteins which do not become biologically functional until in their hydroxylated forms. These hydroxylation-dependent proteins are, for instance, the collagens, the ribosomal initiation factor eIF-5A, and LTBP, the chaperone for synthesis of bioactive TGF-B. If their hydroxylation is suppressed by inhibition of the enzymes which catalyze this reaction, i.e. prolyl 4-hydroxylase, deoxyhypusyl hydroxylase, and aspartyl/asparaginyl hydroxylase, respectively, these proteins are rendered unable to function.

As the functions of these hydroxylation-dependent proteins converge in the clinical disease group of fibrotic and fibroproliferative conditions, the protein hydroxylase inhibitors of Formula (1) may be suitable instruments to control and treat such conditions pharmacologically. Therefore the spirit and scope of the appended claims should not be limited to the description and the preferred versions contain within this specification.

CLAIMS

What is claimed is:

1. A therapeutic composition comprised of the compound of formula (I) and derivatives thereof (salts, tautomeric forms),



(I)

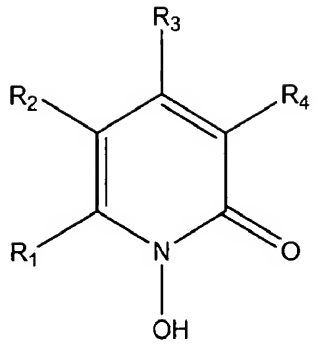
wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

2. Wherein said compound is useful in inhibiting viral replication.

3. Wherein said compound is useful in treating a patient infected with a virus.

4. A method of treating cells infected with a virus comprising:

   administering to eukaryotic cells a composition (compound, creams, lotions, tables, sustained release, intravenous and excipients) comprising the compound of formula (I) and derivatives thereof (salts, tautomeric forms),



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wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms; and

wherein said cells include transcribed viral mRNA, said viral mRNA capable of being transported by an eIF-5A (SEQ ID NO:3) protein of said cells from a nucleus of said cells.

5. The method of claim 1 wherein one or more of said cells are present in a tissue.

6. The method of claim 1 wherein one or more of said cells are present in an individual.

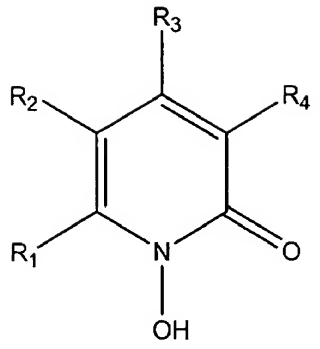
7. The method of claim 1 wherein said viral mRNA is transported to polysomal ribosomes of said cell.

8. The method of claim 1 wherein said virus is a lentivirus or retrovirus.

9. The method of claim 1 wherein said administration includes cycled high dose pulse administrations of said compound.

10. A method of inhibiting viral replication in cells comprising:

administering to eukaryotic cells or tissues including said cells, a composition (creams, lotions, tables, sustained release, intravenous and excipients) including a compound of formula (I) and derivatives thereof (salts, tautomeric forms),



(I)

wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms, and

wherein said compound interrupts/interferes with the formation of eIF-5A (SEQ ID NO:3) protein capable of bonding with a viral mediator (Rev (SEQ ID NO:1)/Rex (SEQ ID NO:2)) protein (proteins encoded by lentiviruses and other complex retrovisus which mediate the nucleocytoplasmic transport of intron-containing, viral pre-mRNAs with the transport protein eIF-5A (SEQ ID NO:3)) within said cells,

11. The method of claim 7 wherein the mediator protein that interacts with the viral mRNA is Rev (SEQ ID NO:1) or Rex (SEQ ID NO:2).

12. The method of claim 8 wherein the Rev (SEQ ID NO:1) protein interacts with the RRE nucleotide sequence of the viral mRNA.

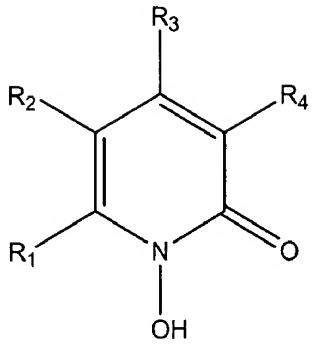
13. The method of claim 8 wherein the Rex (SEQ ID NO:2) protein interacts with the RxRE nucleotide sequence of the viral mRNA.

14. The method of claim 7 wherein the compound interferes with the deoxyhypusine hydroxylase mediated formation of a hydropusine residue on said eIF-5A (SEQ ID NO:3) protein.

15. The method of claim 7 wherein said administration includes cycled (high) dose (pulse) administrations of said composition.

16. A method of modifying apoptosis in cells comprising:

administering to eukaryotic cells, tissues, or individuals a composition including a compound of formula (I) and derivatives thereof (salts, tautomeric forms),



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wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms; and

wherein said cells include (transcribed) viral mRNA that encode proteins to suppress the apoptosis of said cells (and the compound inhibits the formation of a complex between the viral mRNA and a mediator protein such as Rev (SEQ ID NO:1) or Rex (SEQ ID NO:2) and the transport protein eIF-5A (SEQ ID NO:3))

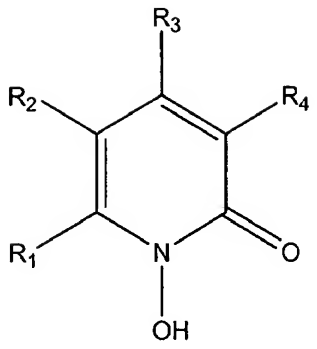
17. The method of claim 13 wherein said compound inhibits the formation of a hypusine residue on a transport protein eIF-5A (SEQ ID NO:3) of said cell, said transport protein interacting with said viral mRNA (to export said viral mRNA from the nucleus to the cytoplasmic polysomes).

18. The method of claim 13 wherein said administration includes cycled high dose pulse administrations of said compound.

19. A method of treating cells infected with a virus comprising:

identifying eukaryotic cells having a virus that uses the eIF-5A (SEQ ID NO:3) cellular protein to transport viral m RNA from the nucleus of said cells to the cytoplasmic polysomes (includes ribosomes) of said cells; and

administering to said cells a compound of formula (I) and derivatives thereof (salts, tautomeric forms),



(I)

wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

20. The method of claim 16 wherein one or more of said cells are present in a tissue.

21. The method of claim 16 wherein one or more of said cells are present in an individual.

22. The method of claim 16 wherein said viral mRNA is transported to polysomal ribosomes of said cell.

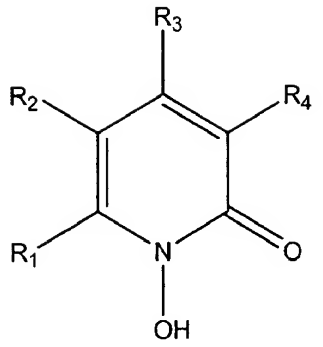
23. The method of claim 16 wherein said virus is a lentivirus or retrovirus..

24. The method of claim 16 wherein said administration includes cycled (high) dose (pulse) administrations of said compound.

25. A method of treating cells infected with a virus comprising:

identifying eukaryotic cells at risk of being infected with a virus that uses the eIF-5A (SEQ ID NO:3) cellular protein to transport viral m RNA from the nucleus of said cells to the cytoplasmic polysomes (includes ribosomes) of said cells; and

administering to said cells a composition including a compound of formula (I) and derivatives thereof (salts, tautomeric forms),



(I)

wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

26. The method of claim 22 wherein one or more of said cells are present in a tissue.

27. The method of claim 22 wherein one or more of said cells are present in an individual.

28. The method of claim 22 wherein said viral mRNA is transported to polysomal ribosomes of said cell.

29. The method of claim 22 wherein said virus is a lentivirus or retrovirus..

30. The method of claim 22 wherein said administration includes cycled high dose pulse administrations of said compound.

31. A method of treating cells infected with a virus comprising:

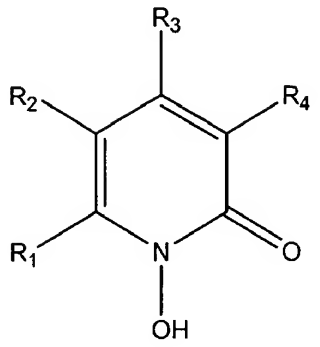
    administering to eukaryotic cells having a foreign RNA in a nucleus of said cells a composition (compound, creams, lotions, tables, sustained release, intravenous and excipients) comprising the compound of formula (I) and derivatives thereof (salts, tautomeric forms).

32. A method of treating HIV in a patient comprised of cycled pulse therapy with a compound of formula (1) to thereby cause selective ablation of infected cells.

33. The method of Claim 32 wherein the cycled pulse therapy is a cycled high dose therapy.

34. The method of Claim 32 wherein 1-hydroxy-2 pyridone is cycled in high dose therapy.

35. A composition comprising: a compound of formula (1)



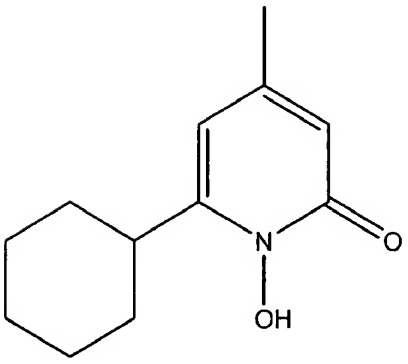
(I)

wherein R_1, R_2, R_3, and R_4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms an aryl aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms; and

inhibiting at least a portion of deoxyhypusine hydroxylase present in said cells.

ABSTRACT OF THE DISCLOSURE

The present invention discloses compounds and pharmaceutical compositions derived from 1-hydroxy-2-pyridines which are highly effective at inhibiting the formation of the hypusine residue in cellular eIF-5A precursor proteins. The invention further relates to methods of using such compounds and pharmaceutical compositions therefrom for inhibiting or preventing the nucleocytoplasmic transport of viral polynucleotides from the nucleus of infected cells to cytoplasmic polysomes for translation. Such inhibition caused by the 1-hydroxy-2-pyridones compounds of the present invention can cause a dose-dependent initiation of apoptosis in the virally infected cells.



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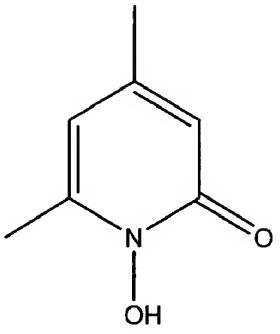

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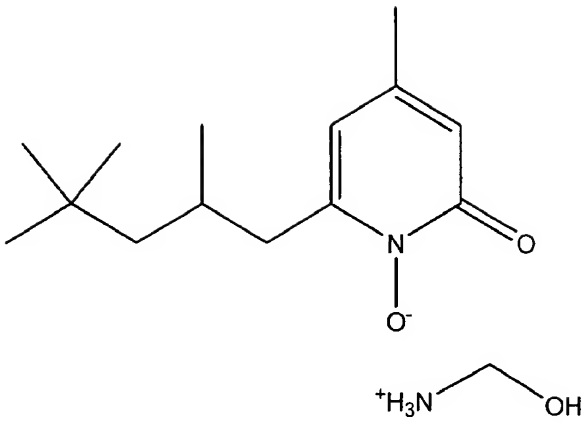

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FIG. 1